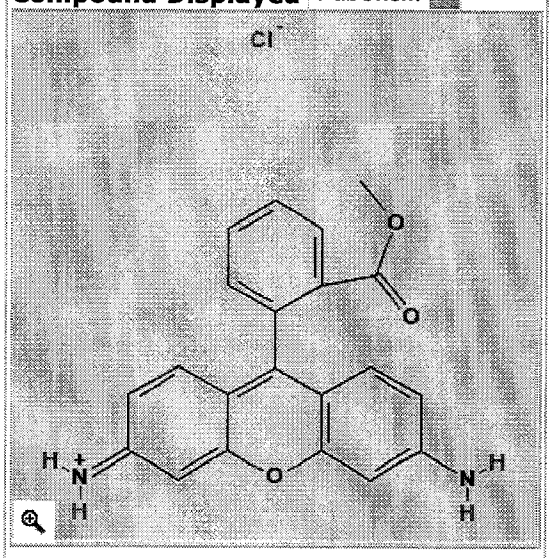
EXHIBIT B

Rhodamine 123 - Substance Summary

(SID: 50063483)

A fluorescent probe with low toxicity which is a potent substrate for P-glycoprotein and the bacterial multidrug efflux transporter. It is used to assess mitochondrial bioenergetics in living cells and to measure the efflux activity of P-glycoprotein in both normal and malignant cells. (Leukemia 1997;11(7):1124-30)

Compound Displayed PubChem



Compound ID	**65217**
Molecular Weight	380.82428 [g/mol]
Molecular Formula	$C_{21}H_{17}ClN_2O_3$
H-Bond Donor	2
H-Bond Acceptor	5

Table of Contents

Links

Chemical Structure Search

Drug and Chemical Info from MeSH: (Total:1)

Rhodamine 123

Pharmacological Action

Fluorescent Dyes

Classification

Heterocyclic Compounds
Heterocyclic Compounds, 3-Ring
Xanthenes
Rhodamines
Rhodamine 123

PubMed Choose by Subheadings:

administration and dosage	adverse effects	analogs and derivatives
analysis	antagonists and inhibitors	blood
chemistry	diagnostic use	isolation and purification
metabolism	pharmacokinetics	pharmacology
therapeutic use	toxicity	urine

PubMed MeSH Keyword Summary

Data Source:

Depositor: LeadScope
External ID: LS-162564

Depositor-Supplied Synonyms: (Total: 8)

Sort: Weight

Rhodamine 123
EINECS 263-687-8
RH 123
LS-162564
3,6-Diamino-9-(2-(methoxycarbonyl)phenyl)xanthylium chloride
Xanthylium, 3,6-diamino-9-(2-(methoxycarbonyl) phenyl)-, chloride
2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester monohydrochloride
Benzoic acid, 2-(6-amino-3-imino-3H-xanthen-9-yl)-, methyl ester, monohydrochloride

Properties Computed from Structure:

Molecular Weight	380.82428 [g/mol]
Molecular Formula	$C_{21}H_{17}ClN_2O_3$
H-Bond Donor	2
H-Bond Acceptor	5
Rotatable Bond Count	3
Exact Mass	380.09277
MonoIsotopic Mass	380.09277
Topological Polar Surface Area	87.1
Heavy Atom Count	27
Formal Charge	0
Complexity	670
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	0
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2

Descriptors Computed from Structure:

IUPAC Name: [6-amino-9-(2-methoxycarbonylphenyl) xanthen-3-ylidene]azanium chloride
Canonical SMILES: COC(=O) C1=CC=CC=C1C2=C3C=CC(=[NH2+]) C=C3OC4=C2C=CC(=C4)N.[Cl-]
InChI: InChI=1/C21H16N2O3.ClH/c1-25-21(24)15-5-3-2-4-14(15)20-16-8-6-12(22)10-18(16)26-19-11-13(23)7-9-17(19)20;/h2-

11,22H,23H2,1H3;1H/fC21H17N2O3.Cl/h22H;1h/q+1;-1

Substance Info:

SID: 50063483
Deposit Date: 2008-07-11
Modify Date: 2008-07-11

CID: 65217
Create Date: 2005-06-24
Parent CID: 65218
Unique Components: 2 Links

Related Substances:
Same: 9 Links

Similar Substances: 199 Links

ASN1	Display	XML	Display	SDF	Display
	Save		Save		Save

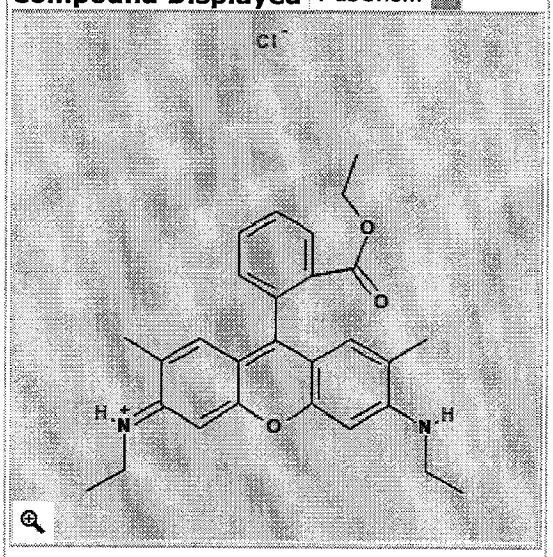
Mitsui Rhodamine - Substance Summary

(SID: 49854251)

RN given refers to HCl

Table of Contents

Compound Displayed PubChem



Compound ID	**13806**
Molecular Weight	479.01034 [g/mol]
Molecular Formula	$C_{28}H_{31}ClN_2O_3$
H-Bond Donor	2
H-Bond Acceptor	5

Drug and Chemical Info from MeSH: (Total:1)

rhodamine 6G

Pharmacological Action
Antineoplastic Agents
Fluorescent Dyes

PubMed

PubMed MeSH Keyword Summary

Links

Chemical Structure Search

Data Source:

Depositor: LeadScope
External ID: LS-22

Depositor-Supplied Synonyms: (Total: 88)

Display: Next 10 | All | Sort: Weight

Mitsui Rhodamine
Rhodamine J
Rhodamine ZH
Rhodamine GDN
Basic Red 1
Rhodamine 4GD
Rhodamine 4GH
Rhodamine 5GL
Rhodamine 6GB
Rhodamine 6GD

Properties Computed from Structure:

Molecular Weight	479.01034 [g/mol]
Molecular Formula	$C_{28}H_{31}ClN_2O_3$
H-Bond Donor	2
H-Bond Acceptor	5
Rotatable Bond Count	7
Exact Mass	478.202321
MonoIsotopic Mass	478.202321
Topological Polar Surface Area	61.5
Heavy Atom Count	34
Formal Charge	0
Complexity	823
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	0
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2

Descriptors Computed from Structure:

IUPAC Name: [9-(2-ethoxycarbonylphenyl)-6-ethylamino-2,7-dimethylxanthen-3-ylidene]-ethylazanium chloride
Canonical SMILES: CCNC1=C(C=C2C(=C1)OC3=CC(=[NH+]CC)C(=CC3=C2C4=CC=CC=C4C(=O)OCC)C)C.[Cl-]
InChI: InChI=1/C28H30N2O3.ClH/c1-6-29-23-15-25-21(13-17(23)4)27(19-11-9-10-12-20(19)28(31)32-8-3)22-14-18(5)24(30-7-2)16-26(22)33-25;/h9-16,29H,6-8H2,1-5H3;1H/b30-24+;/fC28H31N2O3.Cl/h30H;1h/q+1;-1/b

Substance Info:

SID: 49854251
Deposit Date: 2008-07-09
Modify Date: 2008-07-09

CID: 13806
Create Date: 2005-06-24
Parent CID: 13807
Unique Components: 2 Links

Related Substances:
Same: 14 Links

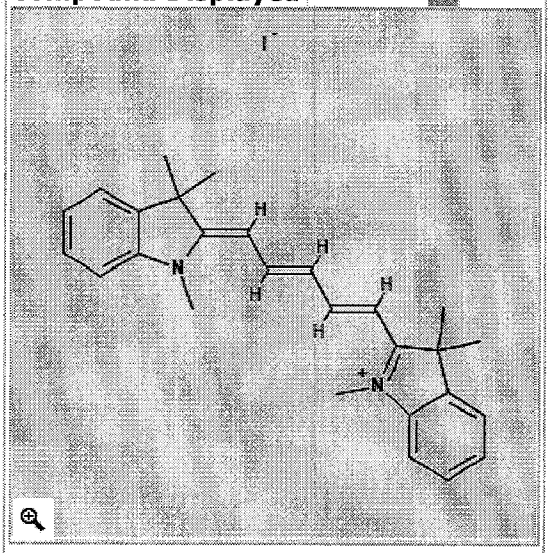
Similar Substances: 332 Links

ASN1 Display Save | XML Display Save | SDF Display Save

HIDC iodide - Substance Summary (SID: 24895800)

Table of Contents
- Data Source
- Synonyms
- Properties
- Descriptors
- Substance Info
- Exports

Compound Displayed PubChem



Compound ID	**6504806**
Molecular Weight	510.45291 [g/mol]
Molecular Formula	$C_{27}H_{31}IN_2$
H-Bond Donor	0
H-Bond Acceptor	2

Data Source:

Depositor: Sigma-Aldrich
External ID: H7636_SIGMA

Depositor-Supplied Synonyms: (Total: 3)

Sort: Weight

HIDC iodide
H7636_SIGMA
1,1,3,3,3′,3′-Hexamethylindodicarbocyanine iodide

Links

Chemical Structure Search

Properties Computed from Structure:

Molecular Weight	510.45291 [g/mol]
Molecular Formula	$C_{27}H_{31}IN_2$
H-Bond Donor	0
H-Bond Acceptor	2
Rotatable Bond Count	3
Exact Mass	510.153192
MonoIsotopic Mass	510.153192
Topological Polar Surface Area	6.3
Heavy Atom Count	30
Formal Charge	0
Complexity	689
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	3
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2

Descriptors Computed from Structure:

IUPAC Name: 1,3,3-trimethyl-2-[(1E,3E,5Z)-5-(1,3,3-trimethylindol-2-ylidene)penta-1,3-dienyl]indol-1-ium iodide
Canonical SMILES: CC1(C2=CC=CC=C2[N+](=C1C=CC=CC=C3C(C4=CC=CC=C4N3C)(C)C)C)C.[I-]
Isomeric SMILES: CC1(C2=CC=CC=C2[N+](=C1\C=C\C=C\C=C/3\C(C4=CC=CC=C4N3C)(C)C)C)C.[I-]
InChI: InChI=1/C27H31N2.HI/c1-26(2)20-14-10-12-16-22(20)28(5)24(26)18-8-7-9-19-25-27(3,4)21-15-11-13-17-23(21)29(25)6;/h7-19H,1-6H3;1H/q+1;/p-1/fC27H31N2.I/h;1h/qm;-1

Substance Info:

SID: 24895800
Deposit Date: 2007-07-16
Modify Date: 2008-01-21

CID: 6504806
Create Date: 2006-05-01
Parent CID: 5477641
Unique Components: 2 Links

Related Substances:
Same: 3 Links
Same, Connectivity: 8 Links

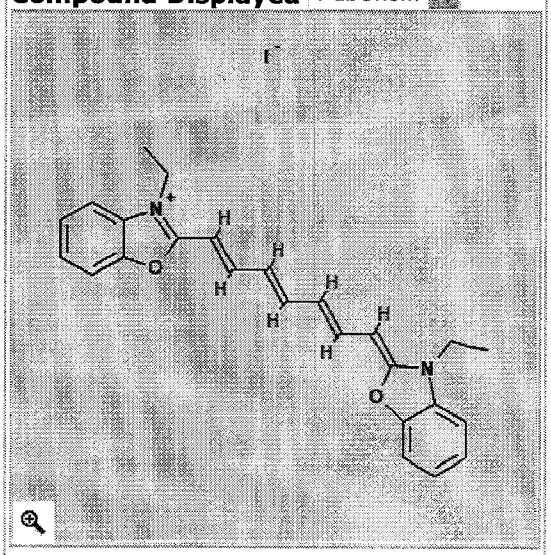
Similar Substances: 787 Links

ASN1	Display / Save	XML	Display / Save	SDF	Display / Save

DOTCI - Substance Summary (SID: 24859444)

Table of Contents
- Data Source
- Synonyms
- Properties
- Descriptors
- Substance Info
- Exports

Compound Displayed PubChem



Compound ID	**5702695**
Molecular Weight	512.38267 [g/mol]
Molecular Formula	$C_{25}H_{25}IN_2O_2$
H-Bond Donor	0
H-Bond Acceptor	4

Data Source:

Depositor: Sigma-Aldrich
External ID: 32448_FLUKA

Depositor-Supplied Synonyms: (Total: 3)

Sort: Weight

DOTCI
32448_FLUKA
3,3'-Diethyloxatricarbocyanine iodide

Links

Chemical Structure Search

Properties Computed from Structure:

Molecular Weight	512.38267 [g/mol]
Molecular Formula	$C_{25}H_{25}IN_2O_2$
H-Bond Donor	0
H-Bond Acceptor	4
Rotatable Bond Count	6
Exact Mass	512.096071
MonoIsotopic Mass	512.096071
Topological Polar Surface Area	29.5
Heavy Atom Count	30
Formal Charge	0
Complexity	601
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	4
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2

Descriptors Computed from Structure:

IUPAC Name: 3-ethyl-2-[(1E,3E,5E,7Z)-7-(3-ethyl-1,3-benzoxazol-2-ylidene)hepta-1,3,5-trienyl]-1,3-benzoxazol-3-ium iodide
Canonical SMILES:
CCN1C2=CC=CC=C2OC1=CC=CC=CC=C3=[N+](C4=CC=CC=C4O3)CC.[I-]
Isomeric SMILES:
CCN\1C2=CC=CC=C2O/C1=C\C=C\C=C\C=C\C3=[N+](C4=CC=CC=C4O3)CC.[I-]
InChI: InChI=1/C25H25N2O2.HI/c1-3-26-20-14-10-12-16-22(20)28-24(26)18-8-6-5-7-9-19-25-27(4-2)21-15-11-13-17-23(21)29-25;/h5-19H,3-4H2,1-2H3;1H/q+1;/p-1/fC25H25N2O2.I/h;1h/qm;-1

Substance Info:

SID: 24859444
Deposit Date: 2007-07-12
Modify Date: 2008-01-18

CID: 5702695
Create Date: 2005-07-19
Parent CID: 2735007
Unique Components: 2 Links

Related Substances:
Same: 5 Links
Same, Connectivity: 6 Links

Similar Substances: 898 Links

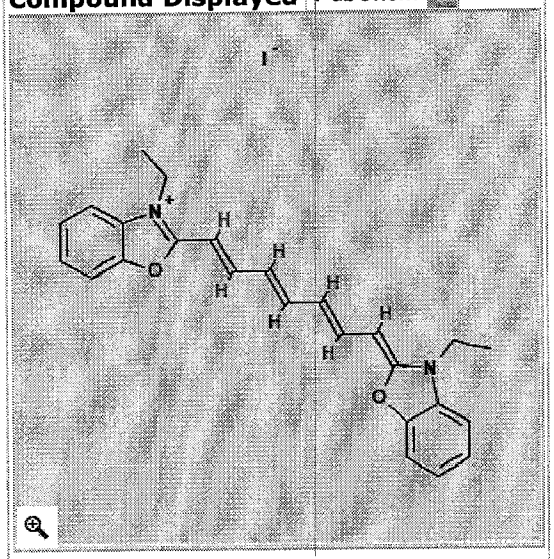
ASN1	Display	Save	XML	Display	Save	SDF	Display	Save

oSeroff

DOTCI - Substance Summary (SID: 24859444)

Table of Contents

Compound Displayed PubChem



Data Source:

Depositor: Sigma-Aldrich
External ID: 32448_FLUKA

Depositor-Supplied Synonyms: (Total: 3)

Sort: Weight

DOTCI
32448_FLUKA
3,3'-Diethyloxatricarbocyanine iodide

Compound ID	**5702695**
Molecular Weight	512.38267 [g/mol]
Molecular Formula	$C_{25}H_{25}IN_2O_2$
H-Bond Donor	0
H-Bond Acceptor	4

Properties Computed from Structure:

Molecular Weight	512.38267 [g/mol]
Molecular Formula	$C_{25}H_{25}IN_2O_2$
H-Bond Donor	0
H-Bond Acceptor	4
Rotatable Bond Count	6
Exact Mass	512.096071
MonoIsotopic Mass	512.096071
Topological Polar Surface Area	29.5
Heavy Atom Count	30
Formal Charge	0
Complexity	601
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	4
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	2

Links

Chemical Structure Search

Descriptors Computed from Structure:

Oseroff

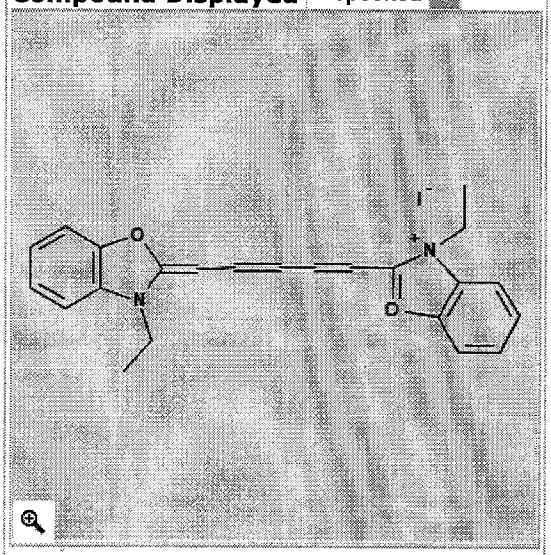
DODCI - Substance Summary

(SID: 661543 Version: 3)

RN given refers to iodide; RN for parent cpd not in Chemline 6/84

Table of Contents
- Drug and Chemical Info
- Data Source
- Synonyms
- Substance Info
- Exports

Compound Displayed Deposited



Drug and Chemical Info from MeSH: (Total:1)

3,3'-diethyloxadicarbocyanine

PubMed

PubMed MeSH Keyword Summary

Links

NLM Toxicology (1)

Chemical Structure Search

Data Source:

Depositor: ChemIDplus
External ID: 014806509

Depositor-Supplied Synonyms: (Total: 12)

Display: Next 2 | All | Sort: Weight

DODCI
Eastman 11219
3,3'-Diethyloxadicarbocyanine
Diethyloxadicarbocyanine iodide
EINECS 238-873-7
3,3'-Diethyloxodicarbocyanine iodide
NSC 270081
3-Ethyl-2-(5-(3-ethyl-2(3H)-benzoxazolylidene)-1,3-pentadienyl)benzoxazolium iodide
3-Ethyl-2-(5-(3-ethyl-3H-benzoxazol-2-ylidene)penta-1,3-dienyl)benzoxazolium iodide
Benzoxazolium, 3-ethyl-2-(5-(3-ethyl-2(3H)-benzoxazolylidene)-1,3-pentadienyl)-, iodide

Substance Info:

SID: 661543

Deposit Date: 2005-08-08

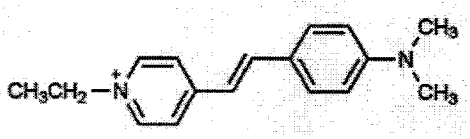
Modify Date: 2006-04-29

ASN1	Display	XML	Display	SDF	Display
	Save		Save		Save

Cyanine

From Wikipedia, the free encyclopedia

Cyanine is a non-systematic name of a synthetic dye family belonging to polymethine group.



Contents

Structure

There are three different types of cyanines:

- *Streptocyanines* or *open chain cyanines*:

  $R_2N^+=CH[CH=CH]_n\text{-}NR_2$ **(I)**

- *Hemicyanines*:

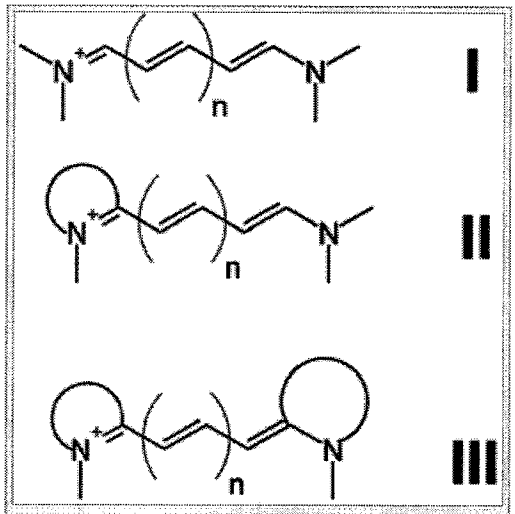
  $Aryl=N^+=CH[CH=CH]_n\text{-}NR_2$ **(II)**

- *Closed chain cyanines*:[1]

  $Aryl=N^+=CH[CH=CH]_n\text{-}N=Aryl$ **(III)**

where two nitrogens are joined by a polymethine chain[2]. Both nitrogens are each independently part of a heteroaromatic moiety, such as pyrrole, imidazole, thiazole, pyridine, quinoline, indole, benzothiazole, etc.

Cyanines were first synthesized over a century ago, and there are a large number reported in the literature.



The Cyanines:
I = Streptocyanines,
II = Hemicyanines,
III = Closed cyanine

Uses

They have many uses as fluorescent dyes. Depending on the structure, they cover the spectrum from IR to UV.

They were originally used, and still are, to increase the sensitivity range of photographic emulsions, i.e., to increase the range of wavelengths which will form an image on the film.

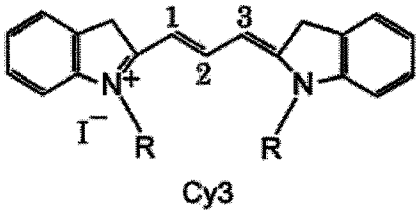
They are used in CD-R and DVD-R media. The ones used are mostly green or light blue in color, and are chemically unstable. This makes cyanine discs unsuitable for archival CD and DVD use, as they can fade and become unreadable in a few years.

Cy3 and Cy5

Cy3 and Cy5 are reactive water-soluble fluorescent dyes of the cyanine dye family. Cy3 possesses red fluorescence, and Cy5 -

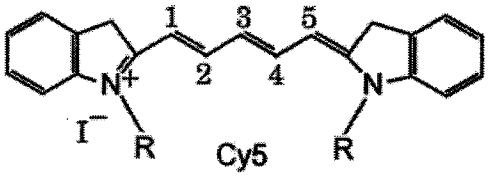
far-red. They are usually synthesized with reactive groups on either one or both of the nitrogen side chains so that they can be chemically linked to either nucleic acids or protein molecules. Labeling is done for visualization and quantification purposes. They are used in a wide variety of biological applications including comparative genomic hybridization and in gene chips, which are used in transcriptomics. They are also used to label proteins for various studies including proteomics.

Nomenclature and Structure



Cy3

Standard chemical names specify exactly the chemical structure of the molecule. The Cy3 and Cy5 nomenclature was first proposed by Ernst, et al.[2] in 1989, and is non-standard, since it gives no hint of their chemical structures. In the original paper the number designated the count of the methines (as shown), and the side chains were unspecified. Thus various structures are designated Cy3 and Cy5 in the literature.



Cy5

The R groups do not have to be identical. In the dyes as used they are short aliphatic chains one or both of which ends in a highly reactive moieties such as N-hydroxysuccinimide or maleimide.

Spectral characteristics

Cy3 is excited maximally at 550 nm and emits maximally at 570 nm, in the red part of the spectrum; quantum yield is 0.15; FW=766.

Cy5 is excited maximally at 649 nm and emits maximally at 670 nm, in the far red part of the spectrum; quantum yield is 0.28. FW=792.

The scanners actually use different laser emission wavelengths (typically 532 nm and 635 nm) and filter wavelengths (550-600 nm and 655-695 nm) to avoid background contamination. They are thus able to easily distinguish between two samples when one sample has been labeled with Cy3 and the other labeled with Cy5. They are also able to quantitate the amount of labeling in either sample.

Cye dye alternatives

Alexa dyes and Dylight dyes can be used interchangeably with Cye dyes in most biochemical applications.

Cy5 ozone susceptibility

In 2003, researchers at Inpharmatics and Agilent reported in *Analytical Chemistry* that microarrays which used Cy5 were susceptible to intermittent data quality decrease caused by environmental ozone. Exposures to ozone levels above 5-10 ppb for 10-30 seconds were reported to decrease the reproducibility of Cy5 microarrays. Much higher levels of ozone (>100 ppb) were required to observe an effect in Cy3 [3].

Applications

Nucleic acid labeling

In microarray experiments DNA or RNA is labeled with either Cy3 or Cy5 that has been synthesized to carry an N-hydroxysuccinimidyl ester (NHS-ester) reactive group. Since, NHS-esters react readily only with aliphatic amine groups, which nucleic acids lack, nucleotides have to be modified with aminoallyl groups. This is done through incorporating aminoallyl-modified nucleotides during synthesis reactions. A good ratio is a label every 60 bases such that the labels are not too close to each other, thus resulting in quenching effects.

Protein labeling

For protein labeling, Cy3 and Cy5 dyes sometimes bear maleimide reactive groups instead. The maleimide functionality allows conjugation of the fluorescent dye to the sulfhydryl group of cysteine residues. Cysteines can be added and removed from the protein domain of interest via PCR mutagenesis.

Cy5, is sensitive to the electronic environment it resides in. Changes in the conformation of the protein it is attached to will

produce an enhancement or quenching of the emission. The rate of this change can be measured to determine enzyme kinetic parameters. The dyes can be used for similar purposes in FRET experiments.

Cy3 and Cy5 are used in proteomics experiments so that samples from two sources can be mixed and run together thorough the separation process[4]. This eliminates variations due to differing experimental conditions that are inevitable if the samples were run separately. These variations make it extremely difficult, if not impossible, to use computers to automate the acquisition of the data after the separation is complete. Using these dyes makes the automation trivial.

References

1. ^ Johannes, H.H.: *Cyanine: Direkte Funktionalisierung, Oligomerisierung, linear und nichtlinear optische Eigenschaften*, Dissertation TU Braunschweig, 2000
2. ^ *a b* Ernst LA, Gupta RK, Mujumdar RB, Waggoner AS. Cyanine dye labeling reagents for sulfhydryl groups. Cytometry. 1989;10(1):3-10. PMID: 2917472
3. ^ Fare TL, Coffey EM, Hongyue D, et.al. Effects of Atmospheric Ozone on Microarray Data Quality. Analytical Chemistry. 2003;75:4672-4675. [1]
4. ^ Unlu M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis. 1997;18(11):2071-7. PMID: 9420172

See also

- N-hydroxysuccinimide
- Maleimide